Micropropagation of Valley Oak Shoots

Kim R. Johnson and Roger F. Walker

Graduate student and assistant professor of forestry, University of Nevada-Reno, Department of Range, Wildlife, and Forestry

Stem segments of valley oak (Quercus lobata Nee) were placed on broad-leaved tree medium (BTM) and Gresshoff-Doy (GD) medium supplemented with 0.55 mg/l of 6-benzylaminopurine (BAP) to observe axillary bud elongation. Both media promoted bud and shoot development, but shoot initiation by explants on GD medium occurred earlier and more abundantly than on BTM medium. - Successful shoot development from axillary buds, with subsequent rooting, will permit production of valley oak planting stock from cryogenically preserved woody tissues of parent trees and this will contribute to the preservation of a species currently in decline throughout much of its native range. Tree Planters' Notes 41(2):27-30; 1990.

Regeneration of cryogenically preserved plant tissues in vitro is an alternative method for the reproduction of species with seeds lacking long-term storage capacity (16). Valley oak (*Quercus lobata* Nee), a riparian and upland alluvial white oak species indigenous to California (7), has significantly declined in number due to agriculture and urbanization (11). Many existing stands are failing to regenerate (6), and attempts to store acorns of the white oaks have often failed (2). Recently, valley oak has been listed as a "species of concern" in California, with the potential to be listed as "threatened" or "endangered" in the near future (14).

In vitro culture of species within the genus *Quercus* has been difficult (9), but some success has been demonstrated. Seckinger *et al*. (13) noted root formation on callus of northern red oak (*Q. rubra* L.) when cultured on Murashige and Skoog (MS) medium supplemented with a-naphthyleneacetic acid (NAA) and benzyladenine (BA). Also, calluses formed on pin oak (*Q. palustris* Muenchh.) cuttings placed on MS medium with various levels of NAA, but neither shoots nor roots were generated (8).

Successful shoot initiation in the oaks has generally been achieved by placing stem segments with one or more axillary buds on media supplemented with low levels of various hormones. Shoot development of northern red oak has been observed on Heller's medium with ammonium sulfate and Gresshoff-Doy (GD) medium (15), MS medium (4), and broadleaved tree medium (BTM) and woody plant medium (WPM) (3), each supplemented with low levels of 6-benzylaminopurine (BAP) or BA. Shoot growth of northern red oak and durmast oak (*Q. petraea* (Matt.) Liebl.) has also been observed on modified WPM and De Fossard medium and aspen culture medium (ACM) supplemented with various concentrations of the hormones BAP, KIN (kinetin), 2iP ([2-isopentenyl]adenine), NAA, indole-3-butyric acid (IBA), and gibberellic acid (GA_3) (10). Bennett and Davies (1) successfully propagated single-node stem segments of Shumard oak (*Q. shumardii* Buckl.) on liquid WPM with BA, and Sato *et al*. (12) propagated epicotyl segments of Sawtooth oak (*Q. acutissima* Carr.) on BTM medium supplemented with BAP.

This study was done to assess the suitability of GD and BTM media augmented with 0.55 mg/l of BAP to promote shoot development from axillary buds of valley oak. Shoot development, with subsequent rooting, will permit woody tissues of parent trees to be regenerated in vitro after cryogenic preservation, resulting in plantlets suitable for use in the production of valley oak planting stock.

C. I. Miller, B. B. Kinloch, and G. E. Dupper of the USDA Forest Service Pacific Southwest Forest and Range Experiment Station; J. R. Seeman and W. S. Johnson of the University of Nevada-Reno; and L. A. Lippitt of the California Department of Forestry assisted with the research. Supported by the Nevada Agricultural Experiment Station and project 619 of the McIntire-Stennis Cooperative Forestry Research Program, University of Nevada, Reno.

Materials and Methods

Stem sections approximately 5 cm in length were taken from vigorous 1-year-old greenhouse-grown valley oak seedlings and all leaves were removed. The sections were cleaned and sterilized ultrasonically and chemically for 3 minutes in a solution of 6% calcium hypochlorite and 0.05% Tween 80 with an adjusted pH of 6.0. The sections were then dipped in a solution of hydrochloric acid at pH 3.5, rinsed three times in sterile water, and cut into segments 1.0 to 1.5 cm long. Only stem tissues that appeared partially lignified were used, as preliminary trials indicated these were less susceptible to damage during sterilization than nonlignified tissues.

After sterilization, stem segments with one or more axillary buds were placed in 100 by 20mm petri dishes containing 35 ml of either GD medium (5) or BTM medium (3) supplemented with 0.55 mg/l of BAP. Each medium also contained 7 g/l of sucrose and 10 g/l of Difco Bacto-agar (Difco Laboratories, Detroit, MI), and the pH was adjusted to 6.0. Both media were autoclaved at 120 °C for 30 minutes before the explants were placed in them. Explants remained in the petri dishes for 1 week but were transferred away from their exudations once daily for the first 3 days.

The explants were then transferred to 22 x 175-mm test tubes containing 15 ml of one of the two culture media prepared as indicated above. One explant was placed in each test tube, 50 explants were set up per treatment, and the explants of each treatment were divided into 10 replications of 5 explants each. After 2 weeks in the test tubes, all explants were placed on fresh media. Explants were grown in an EGC M-12 growth chamber (EGC, Inc., Chagrin Falls, OH) under a combination of metal arc, high-pressure sodium vapor, and incandescent light. The light intensity was attenuated to approximately 300 $\mu E/m^2/s$ (400 to 700 nm) with 50% shade cloth. The photoperiod was 12 hours, and day and night temperatures were set at 23 °C and 16 °C, respectively.

After 28 days, the number of buds induced and the number of shoots on each explant were recorded. The height of each shoot was also measured to the nearest millimeter. The arcsine transformation was performed on the percentages of explants with buds and with shoots, and all differences between treatment means were evaluated with the t test using the Statistical Analysis System (SAS Institute, Inc., Cary, NC).

Results

The bud and shoot productivity of the explants grown on the two media with 0.55 mg/l of BAP differed significantly after 28 days (table 1). Although a greater proportion of the explants grown on BTM medium had buds than those grown on GD medium, a greater proportion of explants on the later medium had produced shoots at this time. A total of 32 buds and 27 shoots were observed on explants cultured on BTM medium and 25 buds and 39 shoots on explants grown on GD medium. The minimum height of the shoots produced on both media was 1 mm, but the largest

Table 1-*Bud and shoot development of valley oak explants grown on broad-leaved tree medium (BTM) and Gresshoff-Doy (GD) medium with 0.55 mg/l of BAP after 28 days*

Medium	Explants With Buds (%)	Explants with shoots (%)	Shoot height (mm)
BTM	64	54	6
GD medium	50	78	6
Level of signifi-cance	*	**	NS

Differences between treatment means were evaluated with the t test; * and ** denote that the means differ at a level of significance of 0.001 <P<0.01 and P<0.001, respectively; NS denotes that P>0.05.

Figure 1—*In vitro shoot development from axillary buds of valley oak after 28 days.* (**A**) *Shoot development of explants cultured on broad-leaved tree medium (BTM) with 0.55 mg/l of BAP.* (**B**) *Shoot development of explants cultured on Gresshoff-Doy (GD) medium with 0.55 mg/l of BAP.* (**C**) *Shoot development of pruned, greenhouse-grown valley oak seedlings. Bars represent 5 mm.*

shoot produced on BTM medium was 19 mm whereas the largest on GD medium was 15 mm. Nevertheless, mean shoot height did not differ between the two treatments.

Overall, the buds and shoots of the explants grown on both media appeared to be healthy and vigorous. Shoots initiated on BTM medium (fig. 1A) and GD medium (fig. 113) were similar in stem diameter, internode length, and number of leaves to shoots originating as sprouts on pruned, greenhouse-grown valley oak seedlings (fig. 1C). Growth rates of shoots produced in vitro also approximated those of shoots occurring on greenhouse-grown seedlings. Leaves produced by shoots in culture were well formed and free of chlorosis and necrosis. In a preliminary test in which valley oak explants were placed on BTM and GD media without BAP, a single small leaf formed on each explant but characteristic shoot development was absent.

Discussion

After 28 days on BTM and GD media supplemented with 0.55 mg/l of BAP, shoot development of valley oak from axillary buds was more prolific on the GD medium. In other studies involving micropropagation of oaks, Vieitez *et al*. (15) reported successful shoot development from axillary buds of *Q. robur* on GD medium supplemented with BAP. However, Chalupa (3) reported BTM medium supplemented with BAP to be more suitable for axillary shoot development of this species than GD medium, as a greater number of taller and more vigorous shoots were produced on the BTM than on the GD medium. In addition, Sato *et al*. (12) found BTM medium with BAP to be satisfactory for shoot development of sawtooth oak. Given that buds were abundant and substantial shoot development also occurred on explants grown on BTM medium in this study, it is possible that this medium would have proven comparable to the GD medium in facilitating shoot development of valley oak if the experiment had been extended. Furthermore, lack of a significant difference between the mean height of the shoots cultured on the two media provides additional indication that both media may be suitable for shoot proliferation of this species in vitro.

The results of this study indicate that both BTM and GD media supplemented with BAP are suitable for the initiation of valley oak shoots from axillary buds, but that shoot proliferation occurs earlier on GD medium. Efforts are currently under way to devise and refine procedures to induce root system development of these shoots. Successful propagation

of viable valley oak plantlets in vitro will permit production of planting stock from cryogenically preserved tissues of this species, and subsequently facilitate the preservation of a species currently in decline in its natural habitat.

Literature Cited

1. Bennett, L.K; Davies, F.T., Jr. 1986. In vitro propagation of *Quercus shumardii seedlings*. HortScience 21:1045-1047.
2. Bonner, F.T. 1981. Oak seedling propagation and production methods: seed technology problems. In: Johnson, P.S.; Garrett, H.E., comps. Workshop on seedling physiology and growth problems in oak planting; 1979 November 6-7; Columbia, MO. Gen. Tech. Rep. NC-62. St. Paul, MN; USDA Forest Service, North Central Forest Experiment Station.
3. Chalupa, V. 1984. In vitro propagation of oak *(Quercus robur* L.) and linden (*Tilia cordata* Mill.). Biologia Plantarum 26:374-377.
4. Favre, J.M.; Juncker, B. 1987. In vitro growth of buds taken from seedlings and adult plant material in *Quercus robur* L. Plant Cell Tissue and Organ Culture 8:49-60.
5. Gresshoff, P.M.; Doy, C.H. 1972. Development and differentiation of haploid *Lycopersicon esculentum* (tomato). Planta 107:161-170.
6. Griffin, J.R. 1976. Regeneration in *Quercus lobata* savannas, Santa Lucia Mountains, California. American Midland Naturalist 95:422-135.
7. Holstein, G. 1984. California riparian forests: deciduous islands in an evergreen sea. In: Warner, R.E.; Hendrix, K.M., eds. California riparian systems: ecology, conservation, and productive management. Berkeley: University of California Press: 2-22.
8. Lineberger, D. 1980. Anatomy of organized structures produced on 'Sovereign' pin oak callus in vitro. Hortscience 15:437 (abstr.).
9. McCown, D.D.; McCown, B.H. 1987. North American hardwoods. In: Bonga, J.M.; Durzan, D.J., eds. Cell and tissue culture in forestry, vol. 3. Dordrecht, The Netherlands: Martinus Nijhoff Publishers: 247-260.
10. Pevalek-Kozlina, B.; Jelaska, S. 1986. In vitro growth and development of oaks (*Quercus robur* and *Q. petraea*). Acta Botanica Croatica 45:55-61.
11. Rossi, R.S. 1980. History of cultural influences on the distribution and reproduction of oaks in California. In: Plumb, T.R., tech. coord. Proceedings, symposium on the ecology, management, and utilization of California oaks; 1979 June 26-28; Claremont, CA. Gen. Tech. Rep. PSW-44. Berkeley: USDA Forest Service, Pacific Southwest Forest and Range Experiment Station: 7-18.
12. Sato, T.; Mori, N.; Saito, A. 1987. In vitro plantlet propagation from epicotyl segments of young seedlings of kunugi (*Quercus acutissima*). Journal of the Japan Forestry Society 69:113-117.
13. Seckinger, G.R.; McCown, B.H.; Struckmeyer, B.E. 1979. Production of anomalous structures in *Quercus rubra* L. callus cultures. American Journal of Botany 66:993-996.
14. Smith, J.P., Jr.; Berg, K. 1988. California Native Plant Society's inventory of rare and endangered vascular plants of California. Spec. Publ. 1, 4th ed. Sacramento, CA: California Native Plant Society.
15. Vieitez, A.M.; San-Jose, M.C.; Vieitez, E. 1985. In vitro plantlet regeneration from juvenile and mature *Quercus robur* L. Journal of Horticultural Science 60:99-106.
16. Zobel, B.J.; Talbert, J.T. 1984. Applied forest tree improvement. New York: John Wiley and Sons.